

that in Finland, despite no difference in HLA-conferred susceptibility (2). Thus, the reason(s) must be linked to environmental factors.

We assessed vitamin D status in the Russian Karelian and Finnish populations to determine whether vitamin D could play a role in the huge difference observed in diabetes incidence. The geographical location in terms of daily sunlight exposure is approximately the same in both populations (~62–66°N). Circulating concentrations of 25-hydroxy (25-OH) vitamin D were analyzed using a commercial enzyme immunoassay kit (Immuno-diagnostic Systems Limited, Boldon, U.K.) in cohorts representing the background population (schoolchildren and pregnant women). The schoolchildren series included 100 subjects from Finland and 100 subjects from Russian Karelia matched for age, sex, and month of sampling (mean age  $\pm$  SD,  $10.9 \pm 1.7$  years, 52% male subjects). Serum samples were collected during the years 1994–2000, and 86% were drawn during March–April. The series of pregnant women included 103 female subjects from Russian Karelia and 172 women from Finland matched for age and date of sampling (mean age  $26.7 \pm 5.3$  years). Samples were collected at the end of the first trimester of pregnancy as a part of the routine prenatal follow-up during the year 2000, and 81% of them were drawn during October–December. The study was approved by the local ethical committees and the Finnish Maternity Cohort steering group. The study was carried out in accordance with the Declaration of Helsinki.

The median serum concentrations of 25-OH vitamin D were approximately the same in both countries among schoolchildren (35.0 in Karelia vs. 39.3 nmol/l in Finland,  $P = \text{NS}$  Wilcoxon test) and pregnant women (28.4 vs. 28.9 nmol/l,  $P = \text{NS}$  by conditional logistic regression test). Sex had no effect on vitamin D status among the schoolchildren. According to the previously suggested cutoff limit for vitamin D deficiency (serum 25-OH vitamin D  $< 25$  nmol/l) (3,4), the proportion of vitamin D–deficient subjects was not higher in Finland compared with Karelia (16 vs. 27% in schoolchildren,  $P = 0.091$ ; 38 vs. 41% in pregnant women,  $P = \text{NS}$ , respectively).

The results suggest that circulating vitamin D concentrations do not differ markedly between Finland and Russian Karelia. Accordingly, vitamin D status may not contribute to the marked differ-

ence in the incidence of type 1 diabetes between the countries, and there must be other factors protecting the Karelian children from developing type 1 diabetes.

Nevertheless, vitamin D substitution remains an important issue in these countries, even beyond infancy, and regular substitution has long been recommended in both countries (5–7), especially during pregnancy, infancy, and the dark months of the year.

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## Soluble Tumor Necrosis Factor Receptor 2 Is Independently Associated With Brachial-Ankle Pulse-Wave Velocity in Nonobese Japanese Type 2 Diabetic Patients

Type 2 diabetes is associated with high mortality and morbidity due to atherosclerosis. Biermen (1) estimated that typical risk factors, including blood pressure, cholesterol, and smoking, can account for no more than 30% of excess cardiovascular risk factor in diabetic patients. Thus, other factors seem to play a role in the progression of atherosclerosis in diabetes.

Aortic stiffness measured by pulse-wave velocity (PWV) is shown to be highly predictive of cardiovascular mortality in type 2 diabetic patients (2). While age and blood pressure are shown to be associated with PWV, age and blood pressure alone do not completely account for the abnormalities of aortic stiffness in type 2 diabetic patients.

Tumor necrosis factor (TNF) system activity seems to be associated with the progression of atherosclerosis in type 2 diabetes. Shai et al. (3) demonstrated that soluble TNF receptor 2 (sTNF-R2) is

strongly associated with risk of coronary heart disease in type 2 diabetic patients. We showed that sTNF-R1 is associated with albuminuria in type 2 diabetic patients (4). To the best of our knowledge, however, it is unclear whether PWV is associated with TNF system activity in type 2 diabetic patients. The aim of the present study, therefore, was to investigate the relationships between PWV and TNF receptors in type 2 diabetic patients.

Eighty-six nonobese Japanese type 2 diabetic patients were enrolled. Their age, BMI, HbA<sub>1c</sub> (A1C), systolic and diastolic blood pressure, and serum creatinine were  $62.8 \pm 1.0$  years,  $22.8 \pm 0.3$  kg/m<sup>2</sup>,  $7.0 \pm 0.1\%$ ,  $136 \pm 2$  mmHg,  $82 \pm 1$  mmHg, and  $0.76 \pm 0.02$  mg/dl, respectively. They had not been treated with insulin. Thirty-four patients were treated with antihypertensive medications. In conjunction with PWV, systolic and diastolic blood pressure, A1C, glucose, lipids, serum creatinine, TNF- $\alpha$ , sTNF-R1, and sTNF-R2 were measured after an overnight fast.

With univariate analysis, PWV was positively correlated to age ( $r = 0.492$ ,  $P < 0.001$ ), diabetes duration ( $r = 0.251$ ,  $P = 0.021$ ), systolic ( $r = 0.595$ ,  $P < 0.001$ ) and diastolic ( $r = 0.248$ ,  $P = 0.022$ ) blood pressure, antihypertensive medication ( $r = 0.268$ ,  $P = 0.013$ ), and the concentrations of sTNF-R1 ( $r = 0.354$ ,  $P = 0.001$ ) and sTNF-R2 ( $r = 0.415$ ,  $P < 0.001$ ). Other variables, including TNF- $\alpha$ , were not associated with PWV. Multiple regression analyses showed that PWV was independently predicted by age ( $F = 15.1$ ), systolic blood pressure ( $F = 31.6$ ), and sTNF-R2 ( $F = 5.2$ ), which explained 49.2% of the variability of PWV. Thus, TNF system activity seems to be associated with atherosclerosis in nonobese Japanese type 2 diabetic patients.

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## Divergent Relationships Among Soluble Tumor Necrosis Factor- $\alpha$ Receptors 1 and 2, Insulin Resistance, and Endothelial Function

Inflammation has the capacity to impair flow-mediated vasodilatation, which is regarded as a causal factor in the development of atherosclerosis (1). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pro-inflammatory cytokine that is also implicated in the pathogenesis of insulin resistance and endothelium dysfunction linked to this event (2). Contradictory effects of TNF- $\alpha$  on endothelial function have been described in different studies (3). Acute intrabrachial TNF- $\alpha$  infusion impairs endothelium-dependent vasodilatation, but TNF- $\alpha$  also enhances protective mechanism (3).

After binding of TNF- $\alpha$  to TNF- $\alpha$  receptors (TNFR1 and TNFR2), a proteolytic cleavage of the extracellular parts of these receptor elicits the soluble forms, named sTNFR1 and sTNFR2 (4).

We aimed to evaluate brachial artery vascular reactivity (high-resolution external ultrasound) and insulin sensitivity (minimal model analysis [5]) in relation with plasma sTNFR1 and sTNFR2 levels (commercially available solid-phase enzyme-amplified sensitivity immunoassays [EASIA]; Medgenix, Biosource Europe, Fleunes, Belgium) in 100 consecutive, apparently healthy, Caucasian men, 70 with normal glucose tolerance (NGT) and 30 with impaired glucose tolerance (IGT), enrolled in a prospective study of insulin sensitivity in Northern Spain.

In multiple regression analysis, serum sTNFR1 independently contributed to endothelium-dependent vasodilatation (EDVD) in subjects with NGT, after adjusting for age, BMI, smoking status, systolic and diastolic blood pressure, and insulin sensitivity ( $\beta = 0.414$ ,  $P = 0.002$ ). In fact, we observed a positive correlation between sTNFR1 levels and endothelium-dependent vasodilatation ( $r = 0.291$ ,  $P = 0.02$ ) (Table 1).

In all subjects as a whole, circulating sTNFR2 was negatively associated with insulin sensitivity ( $r = -0.20$ ,  $P = 0.04$ ) and a trend was observed with EDVD ( $r = -0.190$ ,  $P = 0.058$ ). In IGT subjects, serum sTNFR2 levels correlated negatively with EDVD ( $r = -0.366$ ,  $P = 0.047$ ) (Table 1). The relationship, however, was not significant after adjusting for confounding variables. No association was found between endothelium-independent vasodilatation and circulating sTNFR1 or sTNFR2 levels (Table 1).

This study shows divergent relationships between circulating sTNFRs levels and endothelial function. While sTNFR1 was positively associated with EDVD, opposite relationships regarding sTNFR2 were observed, mainly in subjects with IGT.

Shedding of TNFR1 leads to increased sTNFR1, which antagonizes TNF- $\alpha$  (6). Increased sTNFR1 expression reduced TNF- $\alpha$  bioactivity and protected the myocardium from infarction following ischemia and reperfusion in animal models (7). sTNFR1 might have other protective roles through the stimulation of endothelial cell growth. These antiatherosclerotic mechanisms induced by sTNFR1 are in line with our findings. On the other hand, sTNFR2 levels have been linked to coronary artery disease (8), insulin resistance, and hypertension (5) in concordance with the inverse association between sTNFR2 levels and endothelium